

METHOD FOR ASSEMBLING PCR FRAGMENTS OF DNA

ABSTRACT

[48] A process for assembling a series of DNA fragments generated by PCR into an ordered circular arrangement for replication and genetic work in cells. The PCR fragments are made with a modified nucleotide in the primers that can be removed with a DNA excision repair enzyme to generate a 3' overhang. The 3' overhangs are designed to allow directional annealing and thus sequential PCR fragments can be assembled by annealing the overhangs and subsequent ligation. Sequential addition of PCR fragments is facilitated by growing the chain on a solid support, and the assembled chain can be removed with a site specific recombinase if the first and last primers contain the recombinase site. The circularized assembled fragment can be directly used for cell transformation if the appropriate sequences are included, such as an origin of replication and a selectable marker.